

Case Report: Treatment of Granulomatous Amoebic Encephalitis with Voriconazole and Miltefosine in an Immunocompetent Soldier

Duncan Webster,* Imram Umar, George Kolyvas, Juan Bilbao, Marie-Christine Guiot, Kevin Duplisea, Yvonne Qvarnstrom, and Govinda S. Visvesvara

Saint John Regional Hospital, Saint John, New Brunswick, Canada; Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada; Montreal Neurological Institute, McGill University Health Centre, Montreal, Quebec, Canada; Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada; Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. A 38-year-old male immunocompetent soldier developed generalized seizures. He underwent surgical debulking and a progressive demyelinating pseudotumor was identified. Serology and molecular testing confirmed a diagnosis of granulomatous amoebic encephalitis caused by *Acanthamoeba* sp. in this immunocompetent male. The patient was treated with oral voriconazole and miltefosine with *Acanthamoeba* titers returning to control levels and serial imaging demonstrating resolution of the residual lesion.

INTRODUCTION

Acanthamoeba are ubiquitous free-living amoebas. They have been isolated from a variety of environments throughout the world including soil and airborne dust, as well as fresh brackish and salt waters.¹ *Acanthamoeba* spp. are potential human pathogens and most commonly cause *Acanthamoeba* keratitis (AK). However, this organism also causes granulomatous amoebic encephalitis (GAE), which occurs most commonly in humans with compromised immune systems in the setting of advanced human immunodeficiency virus (HIV) infection, organ transplant, or chronic debilitating illness. The GAE caused by *Acanthamoeba* is a rare and generally fatal illness. Roughly 200 cases have been described and few of these patients have survived.²

A variety of antimicrobial combinations have been used including combinations of pentamidine, sulfadiazine, flucytosine, fluconazole, or itraconazole.³ The combination of trimethoprim-sulfamethoxazole, rifampin, and ketoconazole has been used with success in four pediatric cases.^{4–6} Where intracerebral lesions are observed, surgical resection is generally required.^{7–10} We report the successful treatment of a 38-year-old Canadian soldier with surgical debulking combined with voriconazole and miltefosine.

CASE REPORT

On November 2, 2008 a 38-year-old male soldier with the Canadian Armed Forces developed ringing in his ears. Three days later he noted progressive tinnitus followed by a generalized seizure lasting 15 minutes. He was treated with phenytoin and a computerized tomography (CT) scan revealed a right temporal lobe lesion. Urgent neurosurgical consultation was arranged and on November 13 he underwent a right-sided temporal craniotomy for gross total excision of the right temporal lobe intra-axial mass lesion. The biopsy revealed a dense perivascular and diffuse T-cell inflammatory infiltrate. There was no evidence of clonal B or T-cell lineage and no infectious agent was identified. Immunohistological chemistry was negative for *Toxoplasma gondii*, HHV-8, Epstein Barr virus, and simian virus 40.

The patient showed a slight left facial droop and was maintained on phenytoin. A follow-up magnetic resonance imaging (MRI) on February 17, 2009 showed progression of the lesion and symptomatically the patient was noted to have progressive symptoms including dysarthria, drooling, and recurrent seizure activity. A lumbar puncture was performed, cerebrospinal fluid analysis was unremarkable, and oligoclonal banding was negative. On February 26 the patient underwent reopening of the right craniotomy for debulking and biopsy of the hemispheric mass lesion. The neuropathology (Figure 1A) revealed chronic angiocentric lymphoid infiltrate with florid reactive changes and demyelination. The pathology was not consistent with malignancy, stains were negative for acid fast bacilli, and cultures were negative for bacterial and mycobacterial growth.

The patient's past medical history was remarkable for asthma and hypertension. As a soldier with the Canadian Armed Forces, he had been based in the Atlantic Canadian province of New Brunswick and had served in Bosnia in 1997, Kosovo in 1999, and Afghanistan in 2002.

On March 2, Infectious Diseases consultation was provided and given the unusual cerebral lesion and travel history, echinococcal and *Taenia solium* serology were arranged. Both were negative. In addition a tuberculin skin test was performed that was non-reactive.

On April 2 a follow-up MRI (Figure 1B) showed the lesion to be increasing in size. As tissue pathology had ruled out malignancy and suggested an aggressive form of demyelinating pseudotumor, the possibility of progressive multifocal leukoencephalopathy was raised. The lesion was noted to be atypical, however, and immunohistological studies had been negative for SV40, which essentially ruled out John Cunningham (JC) virus-associated illness. In addition, the patient was noted to be HIV-negative and immunocompetent with an unremarkable T-cell subset analysis, complement levels, and quantitative immunoglobulin screen.

Serology was sent for *Acanthamoeba* and *Balamuthia mandrillaris* and in April the Centers for Disease Control and Prevention (CDC) in Atlanta, GA reported the *Acanthamoeba* titer to be 1:1024 and the *B. mandrillaris* titer to be 1:128. Immunofluorescent studies (Figure 1C) of tissue slides revealed reactivity to anti-*Acanthamoeba* serum. Weak cross-reactivity to anti-*Balamuthia* serum was also observed and on further review of H&E staining, amoeba-like organisms were later identified (Figure 1D). Slide scrapings were used for molecular studies and a positive result for

*Address correspondence to Duncan Webster, Saint John Regional Hospital, Department of Internal Medicine, 400 University Ave. Saint John, N.B., E2L 4L2, Canada. E-mail: Duncan.Webster@HorizonNB.ca

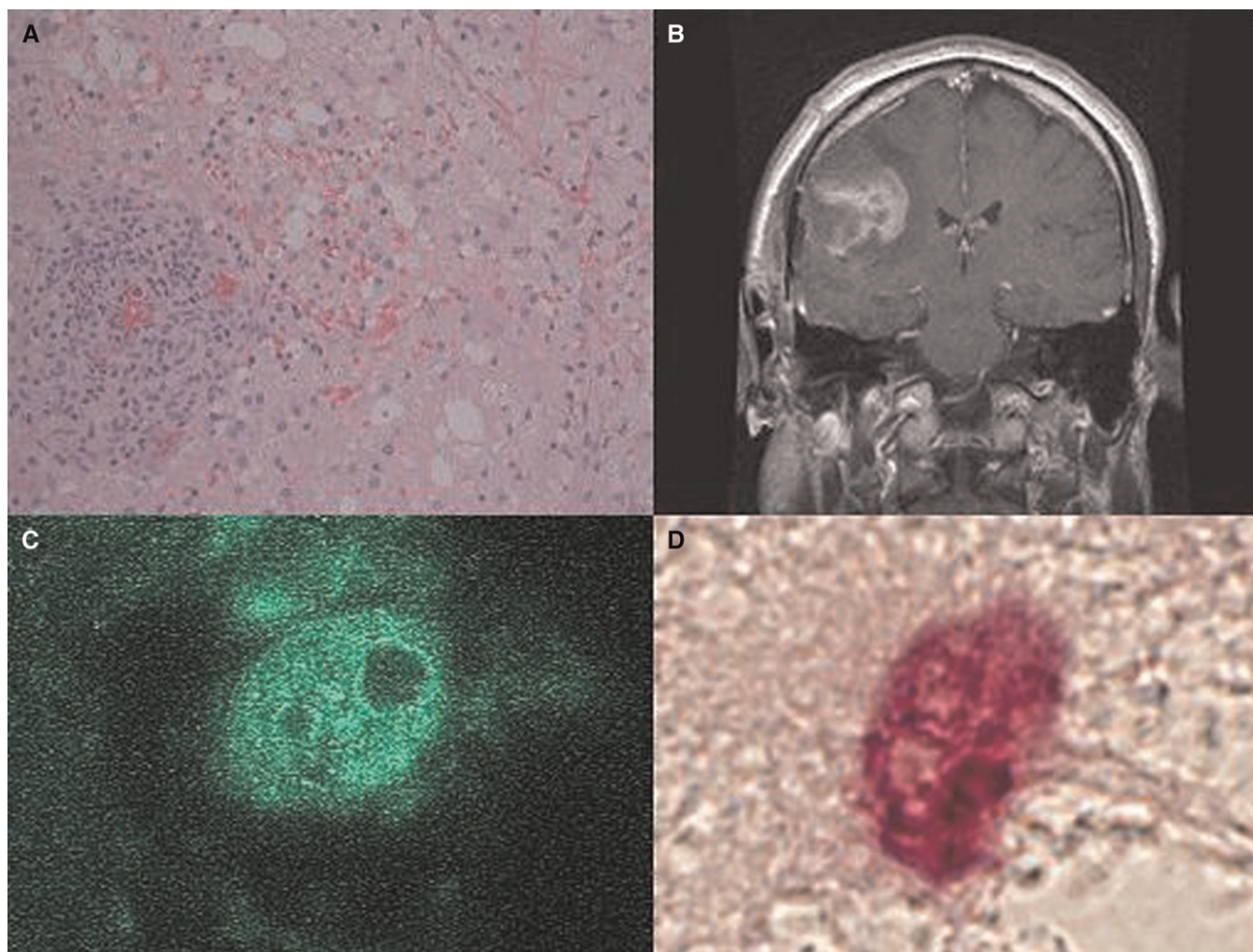


FIGURE 1. **A**, Histopathology of tissue specimen from February 26, 2009 cerebral lesion de-bulking shows chronic angiocentric lymphoid infiltrate with florid reactive changes and demyelination. **B**, April 2, 2009 MRI demonstrating increasing right temporal lobe lesion with irregular enhancement. **C**, Immunofluorescent studies of tissue slides with anti-*Acanthamoeba* serum ($\times 1000$). **D**, H&E stain of brain tissue showing *Acanthamoeba* ($\times 1000$).

Acanthamoeba sp. was obtained by quantitative real-time polymerase chain reaction (Q-PCR) confirming the diagnosis of GAE secondary to *Acanthamoeba* infection.

With this diagnosis, arrangements were made for readmission to the hospital on May 5 for initiation of treatment with voriconazole and miltefosine. At the time of admission, the soldier was noted to be in general good health and on examination exhibited only a mild left facial droop consistent with an upper motor neuron lesion. In addition, he had slightly decreased sensation in the left cranial nerve V distribution and the left forearm with decreased strength of the left hand, specifically finger abduction. A CT of the chest and abdomen was performed to rule out disseminated disease. A repeat MRI of the patient's brain was performed on May 12, which showed radiologic improvements with regards to the lesion and surrounding edema. This was felt to be caused by the aggressive surgical debulking and short course of high-dose steroids during his prior admission.

Before initiating anti-microbial therapy, valproic acid was started while the patient's carbamazepine dose was gradually tapered and phenytoin dose reduced to avoid drug interactions. On May 11 voriconazole 100 mg po bid was initiated.

Because there was a delay in obtaining miltefosine, treatment with this agent was not started until May 21 at which time it was initiated at 100 mg po bid. With voriconazole treatment, mild visual disturbances were noted initially. With initiation of miltefosine, loose stools were experienced over a 6-day period and then settled. Mild nausea with emesis and diarrhea were described after 4 weeks of treatment though this also settled. Monitoring blood work, including liver enzymes and renal function, remained stable. Serum collected on May 21 revealed that the serum antibody titers to *Acanthamoeba* had decreased to normal control levels. An MRI performed on July 3 revealed significantly decreased edema and inflammation at the site of the lesion.

Following 3 months of voriconazole and miltefosine therapy the patient described onset of stomach upset with decreased oral intake. On examination at that time the patient was noted to have a diffuse non-pruritic erythematous rash with associated conjunctival injection. His creatinine had climbed from 69 $\mu\text{mol/L}$ at baseline to 114 $\mu\text{mol/L}$ on August 17. A repeat MRI of his brain on August 20 showed resolution of the lesion. Given the MRI findings in conjunction with the resolution of elevated *Acanthamoeba* titers, the onset of rash,

gastrointestinal upset, and declining renal function, the decision was made to discontinue treatment at the 3-month mark.

With discontinuation of voriconazole and miltefosine the patient's renal function normalized and the rash and gastrointestinal upset resolved. Follow-up examination on October 28 showed improvement of the subtle left facial droop though the patient was left with mild weakness of the left upper extremity. He remained on valproic acid 500 mg po bid as his lone anticonvulsant. On November 16, 2009 the patient did require admission to the hospital in relation to seizure activity at which time his valproic acid was increased to 750 mg po bid. With clinical follow-up, as of August 2011, the patient remained high functioning and in stable medical condition.

DISCUSSION

Acanthamoeba spp. is ubiquitous free living amebas. Based on cyst morphology, which is the traditional basis of identification, roughly 20 species have been identified that are classified into groups I–III.³ Group II is the most abundant and found to be potentially the most pathogenic. By molecular typing by sequencing of the 18S rRNA gene, there appear to be 17 evolutionary lines and as such are classified T1 through T17.^{3,11} *Acanthamoeba* human disease manifestations include AK, granulomatous amoebic encephalitis, sub-acute granulomatous dermatitis, sinusitis, pneumonitis, and disseminated disease. Excluding AK, cases are generally observed in immunocompromised individuals such as those with advanced acquired immunodeficiency syndrome (AIDS), transplant patients, and debilitated individuals with a history of alcoholism, diabetes, or autoimmune disease such as St. Louis encephalitis.² The GAE has been described rarely in the immunocompetent host. Granulomatous amoebic encephalitis is uncommon and it is likely that it goes undiagnosed in many cases. Generally the diagnosis is made post-mortem,

though a small number of confirmed cases of successfully treated GAE and acanthamoebic meningitis have been described. It has been reported that about 150 cases have been published worldwide and < 10 survivors have been identified.¹² Upon further review of the medical literature, we have identified 17 previously reported cases of successfully treated confirmed GAE, acanthamoebic meningoencephalitis, and acanthamoebic meningitis (Table 1).

In the case described in this report, the pathology findings were unique and aided in the eventual diagnosis. The H&E stained sections revealed cortex and white matter with dense angiocentric lymphoid infiltrate, which also surrounded sub-arachnoid blood vessels. The mononuclear cells were immunoreactive for CD45, CD3, CD43, and CD5 identifying them as T cells. Numerous GFAP + reactive astrocytes and abundant macrophages were present with focal necrosis. A panel of special stains including Grocott (GMS) and FITE stain in addition to immunostains for *Toxoplasma*, SV40, HSV 8-11, and EBV were all negative, thus ruling out numerous infectious etiologies. The PCR amplification studies for B- and T-cell gene rearrangements did not show clonal populations of B-cell or T-cell lineage. The differential diagnosis included an aggressive form of demyelinating pseudotumor and given prior reports of GAE in association with other apparent demyelinating processes,²¹ serological testing for *Acanthamoeba* and *B. mandrillaris* was arranged and this ultimately lead to the diagnosis in this case.

No single drug has been found to be effective against systemic acanthamoebiasis, and treatment combinations are largely empirical. Although antimicrobials may show *in vitro* activity, this may not correlate with clinical efficacy. The patient's outcome appears to be impacted by surgical debulking, early initiation of medical therapy, the infective amoebic dose, and the individual's immune status.²

Given data suggesting excellent anti-*Acanthamoeba* *in vitro* activity of voriconazole and miltefosine, in addition to case

TABLE 1
Cases of successfully treated confirmed GAE/meningitis secondary to *Acanthamoeba* sp.*

Reference	Diag.	Species	Year	Country	Age (yrs)	Sex	Ethnicity	ICC	Surgery	Medical treatment
Pan and Gosh ¹³	AME	NA	1970	India	3	M	Indian	NA	No	Amph-B, SDZ, DXS
Pan and Ghosh ¹³	AME	NA	1970	India	0.5	M	Indian	NA	No	Amph-B, SDZ, DXS
Lalitha and others ¹⁴	AM	<i>A. culbertsoni</i>	1983	India	40	M	East Indian	NA	No	Pen-G, chloramphenicol
Ofori-Kwakye and others ⁷	GAE	<i>A. palistinensis</i>	1983	Barbados	7	F	NA	Yes	Yes	Amph-B, KTZ
Karande and others ¹⁵	AME	unspciated	1989	India	0.1	M	NA	NA	No	TMP/SMX, 5-fluorocytosine, SDZ
Sharma and others ¹⁶	AM	unspciated	NA	India	12	F	NA	Yes	No	TMP/SMX
Martinez and others ⁸	GAE	<i>A. castellanii</i>	1998	Spain	33	M	Spanish	No	Yes	SDZ, FLZ
Singhal and others ⁴	AME	unspciated	NA	India	8	F	NA	No	No	KTZ, RIF, TMP/SMX
Singhal and others ⁴	AM	unspciated	NA	India	3	M	NA	No	No	KTZ, RIF, TMP/SMX
Petry and others ¹⁷	AM	genotype T4	2004	Germany	64	F	NA	Yes	No	FLZ, RIF, MTZ, SDZ
Fung and others ⁹	GAE	unspciated	1995	UK	41	M	NA	No	Yes	TMP/SMX, RIF
Aichelburg and others ¹⁰	GAE	genotype T2	NA	Austria	25	M	East Indian	No	Yes	SDZ, FLZ, amph-B, FSN, MFS, AMK
Gupta and others ⁵	AME	unspciated	NA	India	17	M	NA	No	No	KTZ, RIF, TMP/SMX
Sheng and others ¹⁸	GAE	<i>A. castellanii</i>	2008	Taiwan	63	M	Taiwanese	Yes	No	Amph-B, RIF, DXS
Saxena and others ⁶	AM	unspciated	2007	India	15	F	NA	Yes	No	KTZ, RIF, TMP/SMX
Lackner and others ¹⁹	GAE	<i>A. lenticulata</i>	2007	Austria	17	M	Caucasian	Yes	Yes	TMP/SMX, FLZ, RIF, MEPM, LZD, MOXI
Maritschnegg and others ²⁰	GAE	genotype T4	NA	Austria	2	M	NA	No	No	TMP/SMX, FLZ, pentamidine, MFS
Current case	GAE	unspciated	2009	Canada	38	M	Caucasian	Yes	Yes	Voriconazole, MFS

* AME = amoebic meningoencephalitis; AM = amoebic meningitis; AMK = amikacin; Amph-B = amphotericin-B; DXS = dexamethasone; FLZ = fluconazole; FSN = flucytosine; GAE = granulomatous amoebic encephalitis; ICC = immunocompetent; KTZ = ketoconazole; LZD = linezolid; MFS = miltefosine; MEPM = meropenem; MOXI = moxifloxacin; MTZ = metronidazole; NA = not available; Pen-G = penicillin G; RIF = rifampin; SDZ = sulfadiazine; TMP/SMX = trimethoprim/sulfamethoxazole; UK = United Kingdom.

reports using these medications, the patient was initiated on treatment with voriconazole 100 mg po bid on May 11. The antineoplastic agent miltefosine (hexadecylphosphocholine) and the antifungal agent voriconazole represent two promising oral agents with *in vitro* activity against *Acanthamoeba* sp.^{22,23} Both agents penetrate into brain parenchyma and cerebrospinal fluid, and have low toxicity profiles.²²

Voriconazole has previously been used in combination for disseminated cutaneous acanthamoebic infection.²⁴ Miltefosine has also been used in the successful treatment of disseminated disease.¹⁰ This anti-neoplastic agent has also been used to treat visceral leishmaniasis with a degree of clinical success.^{25,26} Additionally, these two agents have been combined in the clinical setting in conjunction with terbinafine in the successful treatment of *Scedosporium prolificans* osteomyelitis.²⁷

In this case, we describe the diagnosis and treatment of an immunocompetent Canadian soldier with GAE secondary to *Acanthamoeba* infection. This patient was successfully treated with a combination of aggressive surgical debulking followed by a 3-month course of miltefosine and voriconazole.

Received February 13, 2012. Accepted for publication June 29, 2012.

Acknowledgments: We thank Stefanie Materniak for assistance with manuscript preparation.

Authors' addresses: Duncan Webster, Saint John Regional Hospital, Department of Internal Medicine, Saint John, Canada, E-mail: Duncan.Webster@HorizonNB.ca. Imran Umar, Saint John Regional Hospital, Department of Laboratory Medicine, Saint John, Canada, E-mail: Imran.UmarDr@HorizonNB.ca. George Kolyvas, Saint John Regional Hospital, Department of Neurosurgery, Saint John, Canada, E-mail: George.Kolyvas@HorizonNB.ca. Juan Bilbao, Sunnybrook Health Sciences Centre, Department of Anatomical Pathology, Toronto, Canada, E-mail: Juan.Bilbao@sunnybrook.ca. Marie-Christine Guiot, Montreal Neurological Institute, Montreal, Quebec, Canada, E-mail: marie.christine.guiot@mcgill.ca. Kevin Duplisea, Department of Pharmacy, Toronto Western Hospital, Toronto, Canada, E-mail: Kevin.Duplisea@uhn.on.ca. Yvonne Qvarnstrom, Division of Parasitic Diseases, National Centre for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: bvp2@cdc.gov. Govinda S. Visvesvara, Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Atlanta, GA, E-mail: gsv1@cdc.gov.

REFERENCES

- Visvesvara GS, Moura H, Schuster FL, 2007. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri* and *Sappinia diploidea*. *FEMS Immunol Med* 50: 1–26.
- Schuster FL, Visvesvara GS, 2004. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int J Parasitol* 34: 1001–1027.
- Abramowicz M (ed), 2007. Drugs for parasitic infections. *Treat Guidel Med Lett* 5 (Suppl): e1–e15.
- Singhal T, Bajpai A, Kalra V, Kabra SK, Samantaray JC, Satpathy G, Gupta AK, 2001. Successful treatment of *Acanthamoeba* meningitis with combination oral antimicrobials. *Pediatr Infect Dis J* 20: 623–627.
- Gupta D, Bakhshi S, Panda GS, 2008. Successful treatment of *Acanthamoeba* meningoencephalitis during induction therapy of childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer* 50: 1292–1293.
- Saxena A, Mittal S, Burnam P, Garg P, 2009. *Acanthamoeba* meningitis with successful outcome. *Indian J Pediatr* 76: 1063–1064.
- Ofori-Kwakye SK, Sidebottom DG, Herbert J, Fischer EG, Visvesvara GS, 1986. Granulomatous brain tumor caused by *Acanthamoeba*. *J Neurosurg* 64: 505–509.
- Martinez MS, Gonzalez-Mediero G, Santiago P, Rodriguez de Lope A, Diz J, Conde C, Visvesvara GS, 2000. Granulomatous amebic encephalitis in a patient with AIDS: isolation of *Acanthamoeba* sp. group II from brain tissue and successful treatment with sulfadiazine and fluconazole. *J Clin Microbiol* 38: 3892–3895.
- Fung KT, Dhillon AP, McLaughlin JE, Lucas SB, Davidson B, Rolles K, Patch D, Burroughs AK, 2008. Cure of *Acanthamoeba* cerebral abscess in a liver transplant patient. *Liver Transpl* 14: 308–312.
- Aichelburg A, Walochnik J, Assadian O, Prosch H, Steuer A, Perneczky G, Visvesvara GS, Aspöck H, Vetter N, 2008. Successful treatment of disseminated *Acanthamoeba* sp. infection with miltefosine. *Emerg Infect Dis* 14: 1743–1746.
- Siddiqui R, Khan NA, 2012. Biology and pathogenesis of *Acanthamoeba*. Available at: <http://www.parasitesandvectors.com/content/5/1/6>. Accessed May 16, 2012.
- Walochnik J, Aichelburg A, Assadian O, Steuer A, Visvesvara G, Vetter N, Aspöck H, 2008. Granulomatous amoebic encephalitis caused by *Acanthamoeba* amoebae of genotype T2 in a human immunodeficiency virus-negative patient. *J Clin Microbiol* 46: 338–340.
- Pan NR, Ghosh TN, 1971. Primary amoebic meningo-encephalitis in two Indian children. *J Indian Med Assoc* 56: 134–137.
- Lalitha MK, Anandi V, Srivastava A, Thomas K, Cherian AM, Chandi SM, 1985. Isolation of *Acanthamoeba culbertsoni* from a patient with meningitis. *J Clin Microbiol* 21: 666–667.
- Karande SC, Lahiri KR, Sheth SS, Nadkarni US, Jain MK, Shah MD, 1991. *Acanthamoeba* meningoencephalitis complicating pyogenic meningitis. *Indian Pediatr* 28: 794–797.
- Sharma PP, Gupta P, Murali MV, Ramachandran VG, 1993. Primary amoebic meningoencephalitis caused by *Acanthamoeba*: successfully treated with cotrimoxazole. *Indian Pediatr* 30: 1219–1222.
- Petry F, Torzewski M, Bohl J, Wilhelm-Schwenkmezger T, Scheid P, Walochnik J, Michel R, Zoller L, Werhahn KJ, Bhakdi S, Lackner KJ, 2006. Early diagnosis of *Acanthamoeba* infection during routine cytological examination of cerebrospinal fluid. *J Clin Microbiol* 44: 1903–1904.
- Sheng WH, Hung CC, Huang HH, Liang SY, Cheng YJ, Ji DD, Chang SC, 2009. First case of granulomatous amoebic encephalitis caused by *Acanthamoeba castellanii* in Taiwan. *Am J Trop Med Hyg* 81: 277–279.
- Lackner P, Beer R, Broessner G, Helbok R, Pfausler B, Brenneis C, Auer H, Walochnik J, Schmutzhard E, 2010. Acute granulomatous *Acanthamoeba* encephalitis in an immunocompetent patient. *Neurocrit Care* 12: 91–94.
- Maritschnegg P, Sovinz P, Lackner H, Benesch M, Nebl A, Schwinger W, Walochnik J, Urban C, 2011. Granulomatous amoebic encephalitis in a child with acute lymphoblastic leukemia successfully treated with multimodal antimicrobial therapy and hyperbaric oxygen. *J Clin Microbiol* 49: 446–448.
- Schuster F, Yagi S, Gavali S, Michelson D, Raghaven R, Blomquist I, Glastonbury C, Bollen AW, Scharnhorst D, Reed SL, Kuriyama S, Visvesvara GV, Glaser CA, 2009. Under the radar: *Balamuthia* amoebic encephalitis. *Clin Infect Dis* 48: 879–887.
- Schuster FL, Guglielmo BJ, Visvesvara GS, 2006. *In vitro* activity of miltefosine and voriconazole on clinical isolates of free-living amoebae: *Balamuthia mandrillaris*, *Acanthamoeba* spp., and *Naegleria fowleri*. *J Eukaryot Microbiol* 53: 121–126.
- Walochnik J, Duchêne M, Seifert K, Obwaller A, Hottkowitz T, Wiedermann G, Eibl H, Aspöck H, 2002. Cytotoxic activities of alkylphosphocholines against clinical isolates of *Acanthamoeba* spp. *Antimicrob Agents Chemother* 46: 695–701.
- Walia R, Montoya JG, Visvesvara GS, Booton GC, Doyle RL, 2007. A case of successful treatment of cutaneous *Acanthamoeba* infection in a lung transplant recipient. *Transpl Infect Dis* 9: 51–54.
- Sundar S, Jha TK, Thakur CP, Bhattacharya SK, Rai M, 2002. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 347: 1739–1746.
- Murray HW, 2012. Leishmaniasis in the United States: treatment in 2012. *Am J Trop Med Hyg* 86: 434–440.
- Kesson A, Bellemore M, O'Mara T, Ellis D, Sorrell T, 2009. *Scedosporium prolificans* osteomyelitis in an immunocompetent child treated with a novel agent, hexadecylphosphocholine (miltefosine), in combination with terbinafine and voriconazole: a case report. *Clin Infect Dis* 48: 1257–1261.